Remarks

Applicants have canceled claim 7 without prejudice.

Applicants have amended the claims 2, 10, 13, and 18 as suggested by the Examiner. Support for the amendment can be found in the independent claims 1, 9 and 17.

Accordingly, no new matter has been introduced by the amendments and their entry is respectfully requested.

Applicants have amended claim 1 to make explicit that which was implicit, namely, that the primer pair that flanks a region of about 100 bp long indeed amplifies a product consisting of about 100 bp. Support for the amendment is found, for example pars [0042] and [0077].

Applicants now turn to the specific remaining rejection and respectfully ask the Examiner to reconsider his position in view of the following.

The Examiner maintained rejections of claims 1, 2, 4-8, 21 and 22 under 35 U.S.C. 103(a) as allegedly being unpatentable over Ruano et al (1990) ("Ruano") in view of Furlong et al (1993) ("Furlong").

As presented in the office response dated April 7, 2008, Ruano does not teach amplifying from a single nucleic acid with three separate and distinct pairs of primers. Ruano only used one primer pair (GR6 and GR5) per amplification reaction to amplify a single DNA segment of 770 bp. Contrary to the Examiner's interpretation, the pair of primers that Ruano used for allele-specific amplification (e.g., GR1/GR3 pair and GR2/GR4 pair) is not primer pairs as the phrase "primer pair" is understood in the art. The primers GR1, GR3, GR2 and GR4 are allele-specific oligonucleotide (ASOs) probes for the dot blot hybridization of the PCR product amplified by GR5/GR6 primer pairs (see Materials and Method section, under subsections "Target for Amplification" and "Dot Blots", and Fig. 2).

Also presented in the office response dated April 7, 2008, the method of Ruano involves a biphasic PCR amplification termed booster PCR (see Materials and Method section, under subsections 'Booster PCR') which is not a step in claim 1. To the extent Ruano suggests possible PCR with multiple primer pairs, Ruano requires **using the 'Booster PCR'** method and does not indicate the overall success rate of doing so with just a single DNA copy. The present claims preclude a booster PCR because only one multiplex amplification is required. The overall

success rate of amplification in Ruano's single primer pair reaction is 24/80 = 35% using the 'Booster PCR' method (p. 6299, col. 1). From the reference, it is not possible to predict whether multiplex PCR without using the 'Booster PCR' method would work when using a single DNA copy as template or whether such a method would even have a similar overall success rate. More likely, one skilled in the art would predict that overall success rate would be lower, given the problems with amplification of single nucleic acid molecule dilutions with even one primer pair, not to mention two or more primer pairs one. Thus there is no motivation even to try. The fact that the Applicants have here shown that multiple primer pairs can be **reliably used** in the same reaction **without such additional booster PCR** is surprising, not to mention the increased efficiency which is completely unexpected as discussed below in more detail, and certainly, there is a significant difference between the efficiency of the method as described in Ruano and the present claims (see, e.g., Figure 3, which shows results from a 5-plex amplification reaction).

In the March 5, 2009 Office Action, the Examiner stated that the Applicants' haplotyping efficiency with 3 SNPs is lower that that disclosed by Furlong, therefore alleging that the assertion of unexpected results of increased genotyping efficiency with claimed method presented in the office response dated Dec 17, 2008, is unfounded.

Applicants respectfully disagree and submit that the "discrepancy" in haplotyping efficiencies is due to the different methods of calculating haplotyping efficiency.

Page 20 (¶083) of the specification teaches the computation of percentage of failed assays (no calls for either SNPs) by counting all failed assays divided by the **total number of assays** performed. Likewise, the percentages of incomplete (missing SNPs), successful (all three SNPs of three loci) or both alleles assays (due to double copies of DNA) are calculated the same way except that the data from those individuals with homozygous haplotypes are excluded. In Table 1, on page 20, the haplotyping of a heterozygous individual is shown. A total of 12 replicates were performed. Repeat #1, 2, 6, 8, 11 and 12 were successful assays. Using the calculation method, as described *supra*, the percentage of successful haplotyping (i.e. "haplotyping efficiency") is 6/12 = 50%. In Fig. 2, the percentage of successful haplotyping is ~ 40-45% from a total of 54-144 individuals. This number would likely be higher if the homozygous haplotypes were included.

The table below summarizes the computations of percentage of successful haplotyping (amplification of three or four SNPs) of the data in Table 1 (for individual Sp-5) and Table 2 (for individual Sp-6) in Furlong using the **total number of assays**, the total number of informative PCR assays or total of number reaction with at least one successful PCR amplification.

	Table 1 (Sp-5) Percentage successful haplotyping (# of successful assays with 3 SNPs = 126)	Table 2 (Sp-6) Percentage successful haplotyping (# of successful assays with 4 SNPs = 47)
Using Total # of assays	126/348 = 36%	47/176 = 27%
Using Total # of informative PCR assays (reactions with two or more amplified SNPs minus both alleles)	126/199 = 63%	47/92 = 51%
Using total of reaction with at least one successful PCR amplification	126/277 = 45%	47/128 = 36%

Using the total number of assays, the calculated efficiency of Furlon's data is ~27-36%, which is significantly lower that of the claimed method. Moreover in Ruano, the success efficiency is 24/80 = 35% (p. 6299, col. 1) for a **single amplification using the 'Booster PCR' method**. Therefore, the Applicants submit that, contrary to the Examiner's allegation, there is, indeed, an unexpected increase in genotyping efficiency which the combination of Ruano and Furlong does not allow one skilled in the art to predict.

In view of the above, Applicants respectfully request the Examiner to reconsider his position with respect to the teachings of Furlong and Ruano, and withdraw the alleged obviousness rejection under 35 U.S.C. 103(a) over Ruano in view of Furlong.

The Examiner also maintained the rejections of claims 1 and 7 under 35 U.S.C. 103(a) over "Ruano" in view of "Furlong" and Ross et. al. ("Ross"). As discussed *supra*, Applicants submit that given the problems with amplification of single nucleic acid molecule dilutions with even one primer pair, not to mention two or more primer pairs, the combination of the teachings of Ruano and Furlong by one skilled artisan would not suggest the unexpected result of increased

genotyping efficiency obtained using the presently claimed method. The teachings of Ross in no way overcome this deficiency. Ross does not assist one skilled in the art to expect increased efficiency for a multiplex PCR reaction without using the 'Booster PCR' method using a single copy DNA as template. All Ross describes is repeating genotyping to resolve genotyping, not increasing efficiency of an amplification reaction using very short i.e. 100 bp fragments.

Accordingly, Applicants respectfully submit that the rejection of claims 1 and 7 under 35 U.S.C. §103(a) should be withdrawn.

The Examiner maintained rejections of claims 3, 9-11, 19 and 20 under 35 U.S.C. 103(a) over "Ruano" in view of "Furlong" and "Ross", and further in view of Drysdale et. al. ("Drysdale").

As presented *supra*, Applicants submit that given the problems with amplification of single nucleic acid molecule dilutions with even one primer pair, not to mention two or more primer pairs, the combination of the teachings of Ruano, Furlong and Ross by one skilled artisan would not suggest the unexpected result of increased genotyping efficiency. The teachings of Drysdale does not overcome this deficiency. Drysdale does not report increased efficiency for a multiplex PCR reaction without using the 'Booster PCR' method using a single copy DNA as template. All Drysdale teaches is general use of haplotypes in the prediction of a phenotypic response to abuterol.

Accordingly, Applicants respectfully submit that the rejection of claims 3, 9-11, 19 and 20 under 35 U.S.C. §103(a) should be withdrawn.

The Examiner maintained rejections of claims 12-18 under 35 U.S.C. 103(a) over "Ruano" in view of "Furlong" and "Ross", and further in view of Rein et. al. ("Rein") and Buckholz et. al. ("Buckholz").

As discussed *supra*, Applicants submit that given the problems with amplification of single nucleic acid molecule dilutions with even one primer pair, not to mention two or more primer pairs, the combination of the teachings of Ruano, Furlong and Ross by one skilled artisan would not suggest the unexpected result of increased genotyping efficiency. The teachings of Rein and Buckholz do not overcome this deficiency. Rein and Buckholz do not report increased

efficiency for a multiplex PCR reaction without using the 'Booster PCR' method using a single copy DNA as template. All Rein teaches are methods for identification of 5-methylcytocine and related modifications in DNA genomes, while Buckholz provides the specific loci and positions for this DNA methylation.

Accordingly, Applicants respectfully submit that the rejection of claims 12-18 under 35 U.S.C. §103(a) should be withdrawn.

The Examiner rejected of claims 23 under 35 U.S.C. 103(a) as allegedly being unpatentable over "Ruano" in view of "Furlong" and "Ross", and further in view of Gerhard et. al. ("Gerhard").

As discussed *supra*, Applicants submit that given the problems with amplification of single nucleic acid molecule dilutions with even one primer pair, not to mention two or more primer pairs, the combination of the teachings of Ruano, Furlong and Ross by one skilled artisan would not suggest the unexpected result of increased genotyping efficiency. The teachings of Gerhard do not overcome this deficiency. Gerhard do not report increased efficiency for a multiplex PCR reaction without using the 'Booster PCR' method using a single copy DNA as template. All Gerhard teaches is a β-globin haplotype with positions that are 15-20 kilo bases pairs apart.

Accordingly, Applicants respectfully submit that the rejection of claim 23 under 35 U.S.C. §103(a) should be withdrawn.

In view of the claim amendments and the arguments set forth *supra*, Applicants respectfully submit that all claims are in condition for allowance. Early and favorable action is respectfully requested.

In the event that any additional fees are required, the PTO is authorized to charge our deposit account No. 50-0850.

Date: August 20, 2009

Respectfully submitted,

/Leena H. Karttunen/

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